

CLAIMS

1. A DNA construct containing two different coding sequences of a fertility restorer gene, both of which encode the same protein product, the first of said sequences being a naturally occurring wild type gene sequence and the second of said sequences being a modified sequence generated by modification of the wild type sequence using codon degeneracy for expression in crop plants to avoid homology between the modified sequence and the wild type sequence at the DNA and mRNA levels so as to reduce susceptibility to homology-based post-transcriptional gene silencing, said wild type and said modified gene sequences being under the control of different tissue specific promoters, said different tissue-specific promoters having overlapping expression patterns in male reproductive tissues of said crop plants.
2. A DNA construct containing two different coding sequences of a fertility restorer gene, both of which encode the same protein product, the first of said sequences being a naturally occurring wild type gene sequence and the second of said sequences being a modified sequence generated by modification of the wild type sequence using codon degeneracy for expression in crop plants to avoid homology between the modified sequence and the wild type sequence at the DNA and mRNA levels so as to reduce susceptibility to homology-based post-transcriptional gene silencing, said DNA construct comprising:
- (i) a first transcription unit comprising the wild type sequence of the restorer gene under transcriptional control of a first tissue-specific promoter (which is used to express a male sterility gene in corresponding male sterile plants) and fused to a transcription termination signal including a polyadenylation signal,
 - (ii) a second transcription unit comprising a modified sequence of the restorer gene under transcriptional control of a second tissue-specific promoter and fused to a transcription termination signal including a polyadenylation signal, and
 - (iii) a third transcription unit comprising a selectable marker gene under transcriptional control of a strong constitutive promoter and fused to a transcription termination signal including a polyadenylation signal,
- wherein the first and second tissue-specific promoters have overlapping expression

patterns in male reproductive tissues of said crop plants.

3. A construct as claimed in claim 2 wherein the restorer gene is a sequence encoding a protein capable of inhibiting or negating the cytotoxic effects of another protein generated by a lethal gene sequence.

5 4. A construct as claimed in claim 2 wherein the restorer gene is selected from the group consisting of *barstar* and protease inhibitors.

5. A construct as claimed in claim 1 wherein said crop plants are dicotyledonous plants.

10 6. A construct as claimed in claim 1 wherein said crop plants are monocotyledonous plants.

7. A construct as claimed in claim 5 wherein said first wild type gene is a *barstar* gene characterized by the nucleotide sequence as shown in Seq. ID # 1.

8. A construct as claimed in claim 7 wherein said second modified gene is a modified *barstar* gene characterized by the sequence as shown in Seq ID # 3.

15 9. A construct as claimed in claim 2 wherein tissue-specific promoters of the first and second transcription units are selected from the group consisting of TA29, A9, A3, *tap1* and *bcp1*.

10. A construct as claimed in claim 2 wherein the preferred first tissue-specific promoter is TA29 and the preferred second tissue-specific promoter is A9.

20 11. A construct as claimed in claim 2 wherein the selectable marker gene is selected from the group of herbicide resistance-conferring genes consisting of *bar* gene, *ALS* gene and *tfdA* gene, or from the group of antibiotic resistance-conferring genes consisting of *nptII* gene, *hpt* gene and *aadA* gene.

25 12. A construct as claimed in claim 2 wherein the strong constitutive promoter for expression of the *bar* gene is selected from the group consisting of CaMV35S single enhancer promoter, CaMV35S double enhancer promoter, MMV and FMV.

13. A fertility restorer transgenic plant and parts or seeds thereof which contain in their nuclear genome the construct of claim 2.

30 14. The transgenic plant as claimed in claim 13 which is selected from the group consisting of dicotyledonous plants and monocotyledonous plants.

15. The plant as claimed in claim 14 wherein said dicotyledonous plant is *Brassica juncea*.

16. A method to obtain efficient fertility restorer lines in crop plants for hybrid seed production, said method comprising the steps of:

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- i) transforming the nuclear genome of plant cells with a DNA construct containing two different coding sequences of a fertility restorer gene both of which encode the same protein product, the first of said sequences being a naturally occurring wild type gene sequence and the second of said sequences being a modified sequence generated by modification of the wild type sequence using codon degeneracy for expression in dicotyledonous crop plants to avoid homology between the modified sequence and the wild type sequence at the DNA and mRNA levels so as to reduce susceptibility to homology-based post-transcriptional gene silencing, said DNA construct comprising:
 - a) a first transcription unit comprising the wild type sequence of the restorer gene under transcriptional control of a first tissue-specific promoter (which is used to express a male sterility gene in corresponding male sterile plants) and fused to a transcription termination signal, including a polyadenylation signal,
 - b) a second transcription unit comprising a modified sequence of the restorer gene under transcriptional control of a second tissue-specific promoter and fused to a transcription termination signal including a polyadenylation signal, and
 - c) a third transcription unit comprising a selectable marker gene under transcriptional control of a strong constitutive promoter and fused to a transcription termination signal including a polyadenylation signal; wherein the first and second tissue-specific promoters have overlapping expression patterns in male reproductive tissues of said crop plants.
 - ii) regenerating transformed plants from said transformed plant cells,
 - iii) identifying transformed plants having a single copy of the DNA construct,
 - iv) crossing the above single copy plants with male sterile *barnase* lines,
 - v) subjecting the F1 progeny obtained from crosses between *barnase* and *barstar* lines to molecular analysis to identify fertility restored plants, and
 - vi) testing pollen viability of fertility restored plants to determine extent of restoration.
17. A method as claimed in claim 16 wherein said crop plants are dicotyledonous plants, preferably, *Brassica juncea*.
18. A method as claimed in claim 17 wherein said restorer lines in *Brassica juncea* are

generated by *Agrobacterium*-mediated transformation using disarmed Ti plasmid.

19. A method as claimed in claim 17 wherein said transformed plants having a single copy of said DNA construct are identified by Southern hybridization.

5 20. A method as claimed in claim 17 wherein prior to said molecular analysis of said F1 progeny, said F1 progeny are analysed and segregated to identify plants containing the marker gene and said marker gene containing F1 plants are analysed for segregation of male-fertile and male-sterile phenotypes on the basis of pollen production and selfed seed formation.

10 21. A method as claimed in claim 17 wherein subsequent to said testing of pollen viability of fertility restorer plants, the restored plants are self pollinated to obtain F2 progeny.

22. A method as claimed in claim 17 wherein said F2 progeny are analysed under field conditions for segregation of male fertile and male sterile phenotypes to confirm the male sterile-restorer combination.

15 23. A method as claimed in claim 17 wherein the preferred restorer gene is *barstar* gene.

24. A method as claimed in claim 17 wherein said first wild type gene is characterized by the nucleotide sequence as shown in Seq. ID # 1

20 25. A method as claimed in claim 24 wherein said second modified gene is characterized by the sequence as shown in Seq ID # 3.

26. A method as claimed in claim 17 wherein the preferred first tissue-specific promoter is TA29.

27. A method as claimed in claim 17 wherein the preferred second tissue-specific promoter is A9.

25 28. A method as claimed in claim 17 wherein the preferred marker gene is *bar* gene.

29. A method as claimed in claim 17 wherein the preferred constitutive promoter is CaMV35S double enhancer promoter.

30. A method as claimed in claim 16 wherein said crop plants are monocotyledonous plants.

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